November 9, 1949.

Miss Netta Sarow
Antioch College,
Yellow Springs, Ohio.

Dear Miss Sanow:

Our interest in tetrazolium has been confined to its application as an indicator of redox reactions, and indirectly, of bacterial pH changes. I would like to help you in any way that I can, but I am not clear what you have in mind in your project. Would you care to outline it a little more fully?

Although we worked with E. coli, I would think that any bacterium will reduce tetrazolium in a properly buffered medium (pH ca 7) in the presence of an oxidisable substrate. Anaerobic conditions are by no means essential, although oxygen will, of course, compete with tetrazolium as an electron donor. A very prompt reaction can be obtained by merely adding tetrazolium, to a final concentration of about 100 ppm, to a growing culture of E. coli in a buffered nutrient broth.

If I may suggest a problem which you may already have anticipated, I found the localization of the tetrazolium into a large polar granule very intriguing. It would be important to determine whether this is the result of a localised reduction of tetrazolium, or merely of an accumulation of lipoid material which dissolves the fat-soluble formazan. This might be checked by attempting to stain bacteria with the formazan itself, which can be obtained readily from tetrazolium by reduction with sodium hydrosulfite, followed by an extraction with ether. It would be best to use the formazan in an alcoholic solution, much as Sudan III or Sudan Black. I think it would also be very interesting to watch vitally stained cells divide to determine whether the stained granule is distributed to the daughter cells.

Please let me know if I can be of any further help. I will be pleased to hear of your results.

Sincerely,

Joshua Lederberg
Assistant Professor of Genetics

P. S. I hope I have your name right. Your signature was almost illegible.